THE EFFECT OF FOLLICLE STIMULATING HORMONE ON THE MITOTIC ACTIVITY OF THE CELLS OF THE GONADS OF IMMATURE MICE

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CHAPTER I

INTRODUCTION

The effects of follicle stimulating hormone on the gonads of mature and immature animals have been the subject of extensive clinical and experimental investigations. The concept of hypophyseal gonadotropins arose from studies upon the effect of administering fresh anterior pituitary tissue to immature rodents. It was observed that such implants caused follicular growth and luteinization in the ovary of the female. Rubinstein ('38) reported that the anterior pituitary-like hormone stimulated the tubular epithelium of the human male to increased proliferation.

In view of these findings, and others cited in the review of literature, this study was undertaken to ascertain whether or not follicle stimulating hormone would bring about an increase in the mitotic activity of the cells of the gonads of immature mice.

CHAPTER II

REVIEW OF LITERATURE

Rubinstein ('38) studied the effect of an anterior pituitary-like hormone on spermatogenesis in the human male. Six adult males were injected intramuscularly three times weekly with 100, 200, and 300 R. U. (rat units), of A. P. L. (anterior pituitary-like hormone of pregnancy urine), until the total dosage had reached 1000 R. U. weekly, for from 5 to 9 weeks. It was observed that, in all cases, the sperm count increased above normal, after from 4 to 7, weeks and remained high from two to 5 weeks after the cessation of injections. He concluded that it appeared that the A. P. L., in addition to its interstitial tissue stimulating effect, also stimulated the tubular epithelium of the testes to increased proliferation. It was also concluded that A. P. L. did not hasten the maturity of the particular germinal cells; instead, it stimulated the proliferation of cells at the stage of maturation in which the testis was.

Nutting ('42) studied the effect of sheep anterior pituitary extract on the ovaries of normal and unilaterally oophorectomized rabbits after they had been injected twice daily for three days with a total of 10 R. U. of pituitary extract. It was reported that at biopsy the ovaries of the control animals showed increased follicular development. On the other hand, the remaining ovaries of the unilateral oophorectomized rabbits were found to have undergone hypertrophy, which was due to increased stroma. Follicular activity was also observed as having been about normal. It was concluded that in the normal animal there may have been a gradation of changes after the injection of the anterior pituitary extract. Due to increased endometrial proliferation and apparently normal follicular activity in the rabbits with one

ovary removed, it was concluded that hypogonadism does not lead to over-stimulation of the remaining ovary by the pituitary.

Hertz and Hisaw ('34) studied the effects of follicle stimulating pituitary extracts on the ovaries of infantile and juvenile rabbits. They reported that the follicle stimulating hormone had no effect on the ovaries of the infantile rabbits, 4-weeks-old. On the other hand, the ovaries of the juvenile rabbits demonstrated marked follicular development when these animals were injected with the same quantity of follicle stimulating hormone. It was concluded by these investigators that the juvenile ovaries were more highly differentiated than the ovaries of the infantile ones. The unresponsiveness of the infantile ovary was due to the absence of atria-containing follicles.

Foster and Fevold ('38) studied artificial follicular development in juvenile rabbits. The investigators injected follicle stimulating hormones and luteinizing hormones, separately and in combination, subcutaneously into juvenile rabbits. It was found that the follicle stimulating hormone alone and in combination with increasing amounts of luteinizing hormone produced follicular development. It was concluded that the follicle stimulating hormone was the only preparation capable of inducing follicular development.

Casida ('35) treated a 36-day-old gilt for 5 days with subcutaneous injections of an aqueous suspension of horse pituitary powder (2.5 gm.). The animal was killed on the 6th day. The ovaries showed no macroscopic evidence of stimulation. However, histological studies of the ovaries showed that the outer peripheral zone of the cortex was greatly crowded with egg nests. The conclusion was drawn that macroscopic evidence of ovarian stimulation with gonad-stimulating extracts does not occur prior to the appearance of vesicular follicles in the ovaries.

Foster and Hisaw ('35) reported that they were successful in inducing experimental ovulation in the immature cat by subcutaneous injections of follicle stimulating hormone, but that ovulation was optimal when follicle stimulating hormone was injected in combination with small amounts of luteinizing hormone. As a result of their findings the investigators concluded that ovulation was not due to the action of follicle stimulating hormone or luteinizing hormone alone; instead, it may be due to the proper synergistic action between the two pituitary gonadotropins.

Bahn, Lorenz, Bennett and Albert ('53) studied the actions of gonadotropins of the pituitary during infancy and early childhood. These investigators prepared homogenates from the hypophyses of two infants (one female and one male) and one child (4-years-old). The homogenate was prepared from the hypophyses of the two infants and injected into hypophysectomized female Wistar rats (30 to 40 days old) in 30 mg. doses. Histological studies of the ovaries failed to reveal increased follicular development. However, when the same amount of the homogenate of the child's hypophysis was injected into another group of rats from the same strain, follicular development was markedly increased, accompanied by ovarian enlargement. They concluded that extracts of the infants' hypophyses did not contain follicle stimulating or luteinizing hormones. It was also concluded that the functional maturity of the anterior lobe of the hypophysis was related to growth of the gland and the production of hormones by the parenchyma. Moreover, it appeared that during childhood the increased follicle stimulating activity preceded increased luteinizing activity of the hypophysis.

Seyle, Collip and Thomson ('35) studied age as a factor in responsiveness to gonadotropic hormones. Twelve immature rats were injected subcutaneously

twice daily with one cubic centimeter of sheep gland pituitary extract. It was noted that vaginal oestrus was manifested within 72 to 96 hrs. and that enlarged luteinized ovaries were produced on the 5th day. It was their conclusion that the follicles became responsive to pituitary extracts when the animals were 18 days old.

Fevold, Hisaw and Hellbaum ('33) injected sexually immature rats twice daily for 5 days with a quarter of a cubic centimeter of follicle stimulating hormone prepared from an aqueous extract of desiccated sheep pituitary powder. The follicle stimulating hormone was found to be very active in stimulating growth of follicles in ovaries of immature rats. They concluded that follicle stimulating hormone alone produced the increased follicular development. Later Fevold ('41) injected immature rats twice daily with 0.25 cc. of follicle stimulating hormone and observed an increase in ovarian weight. He also concluded that the increase in ovarian weight was due to follicular development which was induced by the follicle stimulating hormone administered.

Hellbaum ('36) studied the augmentation of ovary-stimulating action of gonadotropic preparations. He injected immature female rats with extracts of the liver, thyroid, milk, egg white, lemon juice and male human urine in combination with the unfractionated pituitary extract. The augmentative activity of these extracts was determined by the increased weight of the ovaries of the test animals. It was reported that the ovarian weight was markedly increased in each case. He concluded that these extracts do augment the ovary-stimulating action of the unfractionated pituitary extract.

Bischoff ('40) made a comparison between the physiological action of two solutions which contained identical amounts of zinc and a highly purified follicle stimulating hormone fraction. The solutions were adjusted to the

same pH. In one mixture the hormone was insoluble (absorbed); in the other it was soluble (not absorbed). Each preparation was administered to 4 22-day-old rats in a total dose equivalent to one milligram of original powder per rat, given in 4 single daily injections. The results showed that the gonadotropic hormone was not absorbed (did not form an insoluble compound) when zinc was added as the hydroxide. On the other hand, when zinc was added in a soluble form and precipitated as the hydroxide in the presence of the hormone, the hormone was removed from the solution. The preparation which had the hormone in insoluble form (absorbed) produced the large ovaries. The presence of zinc was merely incidental.

In another experiment a highly purified follicle stimulating hormone was injected into 22 and 23-day-old female rats twice daily for 4 days. The dosage administered were 0.5 mg. and 0.10 mg. per cubic centimeter. The results showed that 0.05 mg. of hormone per rat produced roughly a 50% increase in ovarian weight. When the same amount of hormone was administered as an insoluble zinc combination the increase in ovarian weight was 150 to 550% above that produced by the untreated hormone. It was concluded that in the zinc preparation, the hormone was actually precipitated as an insoluble compound. The zinc precipitate, when separated from the supernatant fluid, retained the active material. It was also concluded that augmentation may be produced with the purified follicle stimulating hormone.

Corey ('28) studied the effect of prenatal and postnatal injections of pituitary gland extract in the white rat. It was observed that prenatal injections of pituitary gland extract did not hasten the differentiation of the gonads of the rat. There was no evidence of sexual maturity in either sex until the 10th day of postnatal life, when the effect was most pronounced in

the male. The earliest advance in maturity of the injected females appeared at approximately the 15th day of postnatal life. In the male, there was an apparent increase in tubule length as well as in the width of the tubular wall. There was also an increase in the size of the interstitial cells. It was concluded that the gonads of foetal rats were unresponsive to anterior pituitary extracts from adult rats.

Fluhmann ('33) studied the influence of prolonged administration of gonad stimulating hormones of blood of pregnant women and of sheep anterior pituitary lobe. He injected 23 immature rats with two and five hundredths cubic centimeters of the blood extract for periods of 5, 10, 15 and 20 days. It was observed that the ovarian weights increased with prolonged injections. In another series 23 immature rats were injected with a total dose of two and five hundredth cubic centimeters of an acid extract of sheep anterior pituitary lobe made up so that one cubic centimeter of extract was equivalent to 500 mg. of the dried pituitary powder. The periods of injections were the same as in the first series. It was reported that the ovarian weight increased, upon repeated injections, up to the 5th day, when prolonged injections of anterior pituitary extract no longer brought about an increase in ovarian weight, instead the weight of the ovaries decreased.

The investigator concluded that the blood extract of pregnant women either contained two gonad-stimulating principles which augmented each other, or the ovaries were more sensitive to the gonad stimulating principle contained in it. He postulated that the ovaries were unable to respond completely to excessive stimulation by the anterior pituitary extract in the short period of 5 days.

Lane and Greep ('35) studied the follicular apparatus of the ovary of

hypophysectomized immature rats and the effects of hypophyseal gonadotropic hormone on it. Twenty eight-day-old female rats were hypophysectomized and killed at various post-operative intervals up to 38 days. The ovaries were weighed, fixed and the follicles counted.

Other groups of similarly hypophysectomized females were treated with purified follicle stimulating hormone and luteinizing hormone. These animals were divided into an 'immediate injection' group in which treatment was begun 24 hrs. after the operation and a 'delayed injection' group in which a 9-day post-operative period was allowed to elapse. Injections were made twice daily for three days and the rats killed on the 6th day after the beginning of the treatment.

Those animals which were hypophysectomized and untreated for periods of one to 8 days showed a marked decline in all of the ovarian structures studied. The total follicular content decreased from an average of 373, one day after operation, to 23 follicles after 38 days.

In the rats injected 'immediately' post-operative with follicle stimulating hormone the ovary contained, on the average, 100 more follicles than the operated, uninjected controlled animals. In the rats given 'delayed' follicle stimulating hormone treatment the ovaries contained an average of 94 follicles and the control animals but 58.

Ovaries of the rats treated 'immediately' with purified luteinizing hormone showed total counts of 161, operated uninjected controls 174. The animals given 'delayed' treatment with a similar dosage of luteinizing hormone showed a total follicle count of 66, the controls 58. It was their conclusion that the increase in the total follicle count was largely due to the formation of numerous small primary follicles.

Fevold, Hisaw and Leonard ('31) studied the physiological actions of the anterior pituitary extracts on the reproductive tract of immature female rats, 22 and 25-days-old. The chief criteria used were the ability of the extracts to produce follicular development, luteinization and opening of the vaginal orifice. The animals were divided into two groups, tests and controls. The anterior pituitary lobe extracts were injected subcutaneously in an aqueous solution (0.25 cc. per dose) twice daily for 5 days. At the beginning of the 6th day vaginal smears were taken, the animals killed, the ovaries weighed and fixed for histological studies. They observed that the follicle stimulating hormone, when injected into immature rats, brought about vaginal opening on the third day. On the 5th day vaginal smears showed a state of oestrus. It was also observed that the ovaries had increased in weight when compared with the uninjected controls and contained primarily follicular growth. They reported that the immature rats when injected with the luteinizing hormone of the anterior pituitary lobe failed to produce any ovarian changes. The ovaries of these animals weighed the same as did the ovaries of the untreated controls. They concluded that the anterior lobe of the hypophysis secretes two hormones which act on the ovary: one, a gonad-stimulating hormone, which stimulates follicular growth; another, a luteinizing hormone, which causes lutein growth. It was also concluded that the immature ovary must be stimulated to follicular activity by the gonad-stimulating hormone before a characteristic "mulberry" ovary can be produced.

Rubinstein and Radman ('38) made a comparative study of the gross and microscopic effects of follicle stimulating hormone and anterior pituitary sex hormone on the rat testis. Eighty Wistar rats (30 to 108 days old) were divided into two groups. Each sub-group was further divided into 20 test

animals and 20 controls. Twenty test animals were injected intraperitoneally daily with 10 rat units of the follicle stimulating principle of urine for 10 days. The other group of test animals received similar injections of 10 rat units of sheep anterior pituitary sex hormone for 4 days, then 20 rat units for 6 days. The control animals received no injections. It was reported that F. S. U. (follicle stimulating principle of urine) and anterior pituitary sex hormone produced a significant increase in testicular size of the test animals as compared with the uninjected control animals. It was also reported that sheep anterior pituitary sex hormone as well as follicle stimulating principle of urine stimulated the descent and development of the testes of normal immature and mature male rats. Through histological studies it was found that both hormones had brought about proliferation of the interstitial tissues and of the germinal epithelium of the tubular elements. It was concluded that even though both hormones led to increased interstitial and tubular proliferation, spermatic maturity was not hastened.

Saunders ('47) reported that ovulation could be produced in diestrus mice by the administration of pituitary gonadotropins. Seven hundred mice were divided into three groups. The first group received 0.25 ml. of unfractionated pituitary extract in a single subcutaneous injection. The animals were killed 24 hrs. later and the oviducts examined for swellings under the dissecting microscope. The oviducts were serially sectioned and ova counted. It was reported that unfractionated pituitary extract produced 100% ovulation in the mice which showed cyclic estrous. The second and third groups were similarly treated with 0.25 ml. of follicle stimulating and luteinizing hormones respectively. It was observed that the follicle stimulating hormone rarely induced more than 70 or 80% ovulation. The luteinizing hormone failed to induce ovulation. It was concluded that the unfractionated pituitary extract contained both follicle stimulating and luteinizing hormone which acted on the ovaries synergistically in the induction of ovulation.

CHAPTER III

MATERIALS AND METHODS

Twenty adult mice (14 females, two of which were pregnant, and 6 males) were received from Rockland Farms, New City, New York. The animals were maintained in a constant temperature room at 75°C. on a diet of rabbit ration pellets and received water ad libitum. The follicle stimulating hormone used in this experiment, was obtained in powdered form from the National Biochemical Corporation, Cleveland, Ohio.

Twenty four 22-day-old immature mice (all offsprings of the original 20 animals) were divided into two groups: experimentals and controls. Each group consisted of 6 males and 6 females. Five hundredths gram of follicle stimulating hormone was dissolved in 200 ml. of 0.9% saline solution in order that each millileter of the prepared solution would contain 0.25 mg. of the original powder. The experimental animals received subcutaneous injections of one millileter of the follicle stimulating hormone preparation daily for 6 days. The control animals received similar injections of the same amount of an egg albumin preparation for the same period as the experimental animals. Two animals (one male and one female) from each group were sacrificed daily after the initial injection for periods of 24, 48, 72, 96, 120 and 144 hrs. These intervals of injections were used so that the first animals sacrificed had received only one injection and the last animals sacrificed had received a total of 6 injections. At autopsy the gonads were dissected free of adventitious tissue under a dissecting microscope. All tissues (gonads) were prepared for histological studies by the dioxane method. However, only one gonad (one testis and one ovary) from each animal sacrificed was sectioned at 10 u. and stained with hematoxylin and eosin.

The mitotic activity was determined by counting all mitoses in a section of a known area under an oil immersion lens (97 X). The nuclear density of the section was determined by averaging the number of nuclei counted in several sections of known areas. The area was calculated after calibrating the ocular micrometer with a stage micrometer whose scale measured two millimeters in length and was graduated in tenths of a millimeter. The mitotic activity was then computed according to the following formula (after Wilson and Leduc '47):

$$\frac{\text{Number of mitotically active nuclei}}{\text{area of section X nuclear density of section}} =$$

mitotic activity

CHAPTER IV

EXPERIMENTAL RESULTS

Histological studies of the prepared slides revealed that the mitotic activity of the cells of the gonads of the immature mice were activated when the experimental animals were injected with follicle stimulating hormone. However, the effect was more pronounced in the testis than in the ovary (Figs. 1 and 2). In the testes, the mitotic activity showed a gradual increase from one to 4 days after injections. The majority of the mitotically active cells were observed in the area of the germinal epithelium. From the 4th to the 6th day after injection the mitotic activity showed a marked increase. It was observed that more of the primary and secondary spermatocytes were undergoing mitoses. The greater portion of the mitoses which were observed in the areas of the primary and secondary spermatocytes were adjudged to have been in metaphase, anaphase and telophase stages. The mitotic activity of the cells of the ovaries showed only a slight increase from the first to the 5th day after injections. From the 5th to the 6th day the mitotic activity decreased.

When the experimental tissues were compared with the control tissues it was observed that on the first day post-injection the testis of the experimental animal showed only a slight increase in mitotic activity above that of the control animal (Fig. 1). The ovaries of the experimental animals showed no significant difference from that of the control animals in mitotic activity during the 6-day period of injections. The ovaries of the experimental animals showed only a slight increase in the number of mitotically active cells during the 6-day injection period.

The gonads of the egg albumin injected control animals did not show a significant increase in mitotic activity during the same injection period as the experimental animals. Instead, the mitotically active cells of the gonads of these animals demonstrated a repeated increase and decrease in the number of mitoses observed (Figs. 1 and 2).

The most significant increase in mitotic activity in the testes of the experimental animals over that of the control animals was observed from the 4th to the 6th day post-injection.

The testes of the control animals failed to show a steady increase in mitotic activity (Fig. 1). This was possibly due to the interaction between the anterior pituitary gland and the gonads (check and balance).

The testis of the experimental animal showed 3.61% more mitoses on the 4th day post-injections than that of the control animal. On the 6th day the testis of the experimental animal demonstrated 10.2% more mitoses than did the testis of the control animal.

It was observed that in all cases the average cell count appeared fo fluctuate with the size of the cells, the number of cells undergoing mitosis, and the areas in which the cell counts were made. When the cells were very large or when a large number were observed in metaphase, anaphase or telophase, the total cell count devreased. The total cell count was also less when the areas studied included an ovum of the ovary or the lumen of the seminiferous tubule (Tables 1 and 2).

CHAPTER V

DISCUSSION

According to Rubinstein ('38) and Rubinstein and Radman ('38) follicle stimulating hormone when injected into immature male rats caused cellular proliferation in the testes. The results of the present experiment appeared to corroborate the findings of these investigators. It was demonstrated that follicle stimulating hormone administered in 0.25 mg. dosages at intervals of one, two, three, 4, 5 and 6 days caused an increase in mitotic activity of the testes of immature mice. This increase in mitotic activity might have caused the increase in tubular length and in the width of the tubular wall of the testes as reported by Corey ('28). It was observed that as the total number of injections was increased the mitotic activity also increased.

The ovaries of the experimental animals appeared to have been less responsive to the amount of follicle stimulating hormone administered than the testes were. The mitotic activity of the ovaries increased gradually up to the 5th day post-injection. On the 6th day post-injection the mitotic activity of the ovaries decreased. These observations appeared to agree with the findings of Fluhmann ('33) who reported that prolonged injection of follicle stimulating hormone brought about an increase in ovarian weight up to the 5th day, upon repeated injections, after which the ovarian weight decreased.

It has been reported that the ovaries of immature and mature animals demonstrated follicular growth and development when injected with follicle stimulating hormones (Hertz and Hisaw, '34, Foster and Fevold, '38, Fevold, Hisaw and Hellbaum, '33, Fevold, '41, and Lane and Greep, '35). Based on the results obtained in this investigation the increase in ovarian size and weight cannot be attributed to increased mitotic activity.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. Follicle stimulating hormone did produce an increase in the mitotic activity of the cells of the gonads of immature mice; however, the effect of the injected follicle stimulating hormone was more pronounced in the testes.

2. The inability of the ovary to demonstrate marked mitotic activity was probably due to its unresponsiveness to the follicle stimulating hormone administered within the short period of 6 days.

3. The egg albumin preparation did not appear to enhance proliferation of the cells of the gonads of the immature mice.

4. The mitotic activity of the testes of the control animals appeared, for the most part, to be confined to the germinal epithelium.

LITERATURE CITED

Bahn, Robert C., Mona Lorenz, Warren A. Bennett and A. Albert 1953 Gonadotropins of the pituitary gland during infancy and early childhood. Endocrinology, 52: 605-606.

Bischoff, Fritz 1940 Non-specific augmentation with a highly purified follicle stimulating hormone fraction (Evans). Endocrinology, 27: 554-558.

Casida, L. E. 1935 Prepuberal development of the pig ovary and its relation to stimulation with gonadotropic hormones. Anat. Rec., 61: 389-396.

Corey, E. L. 1928 The effect of prenatal and postnatal injections of pituitary gland in the white rat. Proc. Soc. Exp. Biol. and Med., 25: 498-499.

Fevold, H. L. 1941 Synergism of the follicle stimulating and luteinizing hormones in producing estrogen secretion. Endocrinology, 28: 33-36.

Fevold, H. L., F. L. Hisaw, and A. Hellbaum 1933 Sex hormones of the anterior lobe of the hypophysis. Further purification of a follicle stimulating factor and the physiological effects on immature rats and rabbits. Amer. Jour. Physiol., 104: 710-723.

Fevold, H. L., F. L. Hisaw and S. L. Leonard 1931 The gonad stimulating and the luteinizing hormones of the anterior lobe of the hypophysis. Amer. Jour. Physiol., 97: 291-301.

Fluhmann, C. F. 1933 Comparative studies of gonad-stimulating hormones. II. Influence of length of period of administration of certain extracts. Proc. Soc. Exp. Biol. and Med., 30: 1014-1016.

Foster, Mark A. and H. L. Fevold 1938 The interrelationship of the pituitary gonadotropic hormones in follicular development and ovulation of the juvenile rabbit. Amer. Jour. Physiol., 121: 625-632.

Foster, M. A. and F. L. Hisaw 1935 Experimental ovulation and the resulting pseudo-pregnancy in anoestrous cats. Anat. Rec., 62: 75-93.

Hertz, Roy and F. L. Hisaw 1934 Effects of follicle-stimulating and luteinizing pituitary extracts on the ovaries of the infantile and juvenile rabbits. Amer. Jour. Physiol., 108: 1-13.

Hellbaum, Arthur A. 1936 Augmentation of ovary-stimulating action of gonadotropic preparations. Proc. Soc. Exp. Biol. and Med., 33: 568-570.

Lane, C. E. and R. O. Greep 1935 The follicle apparatus of the ovary of the hypophysectomized immature rat and the effects of hypophyseal gonadotropic hormone on it. Anat. Rec., 63: 139-146.

Nutting, Fellows 1942 Effect of sheep anterior pituitary extract on rabbit ovaries and uterus. Endocrinology, 31: 146.

Rubinstein, H. S. and H. M. Radman 1938 A comparative study of the gross and microscopic effects of follicle stimulating hormone and anterior pituitary sex hormone on the rat testis. Amer. Jour. Physiol., 122: 319-324.

Rubinstein, H. S. 1938 The effect of the anterior pituitary-like hormone on spermatogenesis in the human. Endocrinology, 23: 75-78.

Saunders, Francis J. 1947 Induction of ovulation in the diestrous mouse by gonadotropins. Endocrinology, 40: 1-8.

Seyle, H., J. B. Collip and D. L. Thomson 1935 The age factor in responsiveness to gonadotropic hormones. Proc. Soc. Exp. Biol. and Med., 32: 800-803.

Wilson, J. W. and E. Leduc 1947 Mitotic rate in mouse liver following intraperitoneal injections of liver, kidney and egg yolk. Anat. Rec., 97: 470-494.

TABLE 1

THE AVERAGE NUMBER OF CELLS AND THE MITOTIC ACTIVITY FOR THE EXPERIMENTAL ANIMALS

Hrs. after Injections	Average cell counts/.0144mm.^{2}		Male	Female	Mitotic Activity
	Total	Mitotic			
24	168.3	20.9	v		8.6
24	178.1	2.1		v	0.8
48	158.7	22.0	v		10.0
48	212.1	4.2		v	1.3
72	187.9	33.4	v		12.3
72	189.7	5.1		v	1.8
96	201.7	40.6	v		14.0
96	212.1	5.9		v	1.9
120	188.5	54.5	v		18.7
120	218.2	6.6		v	2.1
144	164.8	55.5	v		23.4
144	199.6	5.2		v	1.8

TABLE 2

THE AVERAGE NUMBER OF CELLS AND THE MITOTIC ACTIVITY
FOR THE CONTROL ANIMALS

Hrs. after Injections	Average cell counts/.0144mm.^{2}		Male	Female	Mitotic Activity
	Total	Mitotic			
24	122.0	13.7	v		7.8
24	209.0	2.7		v	0.9
48	136.0	11.7	v		5.97
48	219.7	2.7		v	0.85
72	174.5	16.2	v		6.4
72	212.1	3.7		v	1.2
96	198.2	21.7	v		7.6
96	207.3	3.3		v	1.1
120	185.7	18.0	v		6.8
120	217.6	2.9		v	1.3
144	184.3	20.5	v		7.7
144	211.6	2.7		v	0.8

PLATE 1

(Explanation of Graph)

(Explanation of Graph)[1]

This is a graph showing the comparative mitotic activity per unit time for the experimental and control males. (0 -- 0 = Experimentals)(X - • - X = Controls).

[1] Ordinate axis shows mitotic activity. Abscissa axis shows length of time in days post-injection.

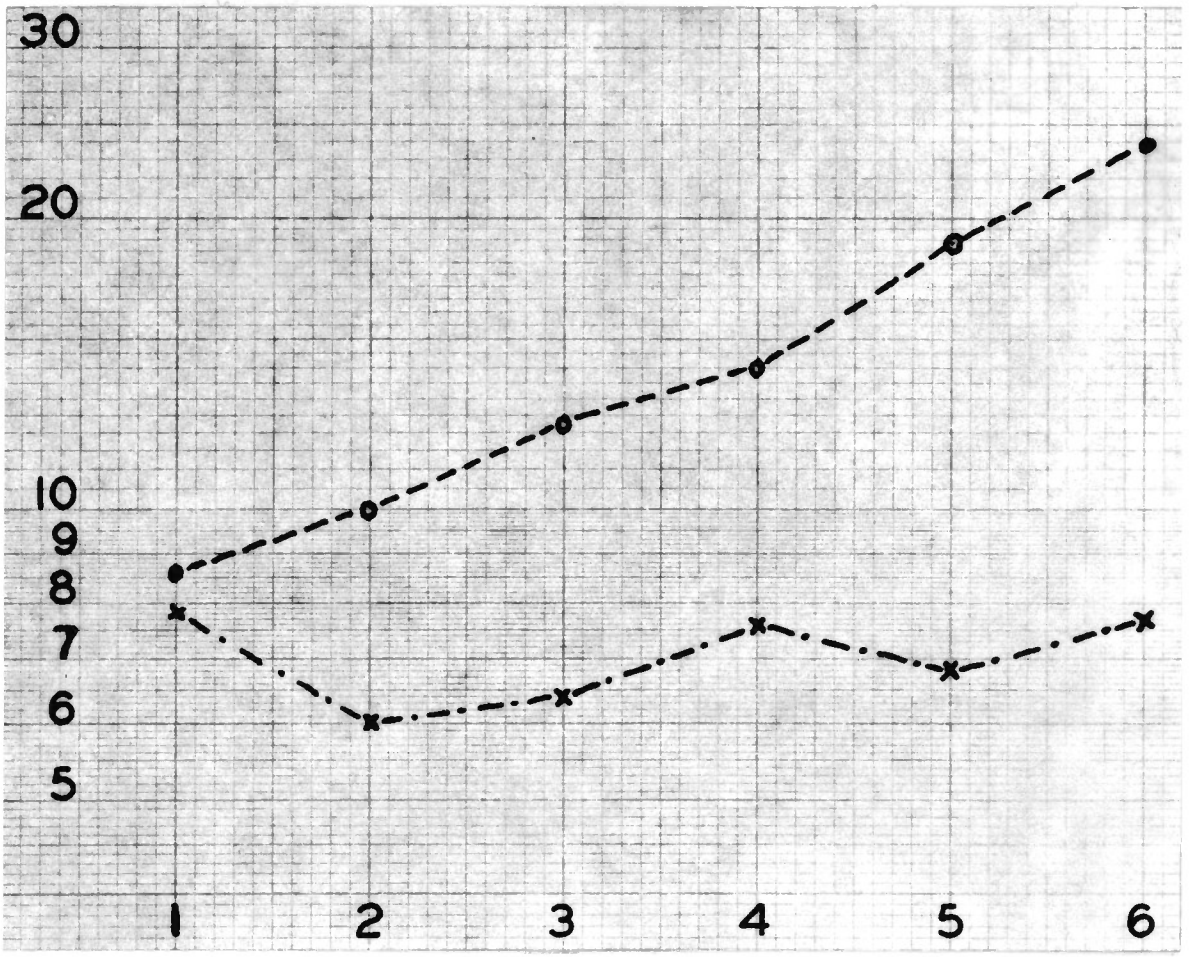
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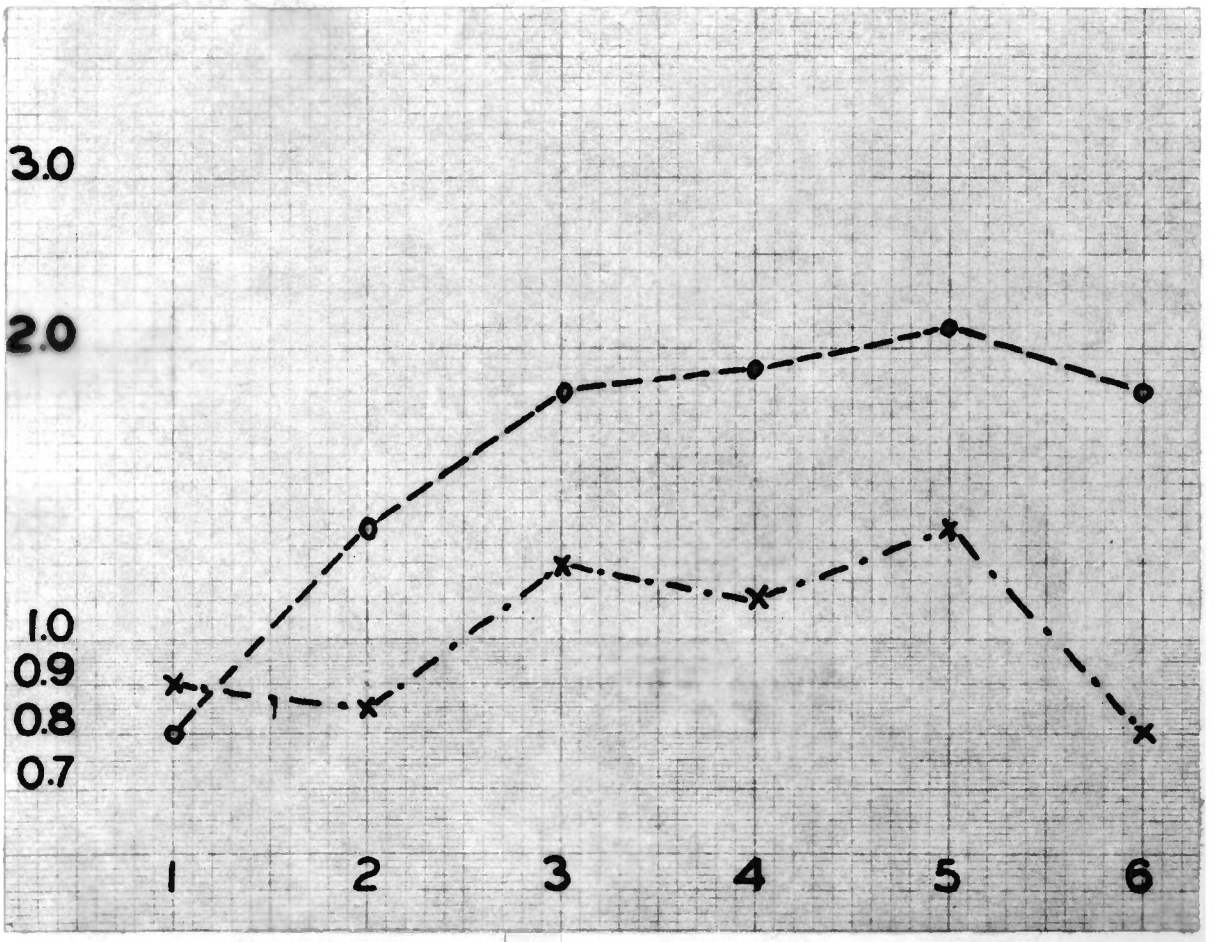
PLATE II

(Explanation of Graph)

(Explanation of Graph)[2]

This is a graph showing the comparative mitotic activity per unit time for the experimental and control females. (O -- O = Experimentals)(X - • - X = Controls).

[2] Ordinate axis shows mitotic activity. Abscissa axis shows length of time in days post-injection.



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